FOUNDATIONONE® LIQUID CDx

PATIENT

TUMOR TYPE
Breast invasive ductal carcinoma (IDC)

REPORT DATE

COUNTRY CODE
CA

ORDERED TEST #

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT	PHYSICIAN	SPECIMEN
DISEASE Breast invasive ductal carcinoma (IDC)	ORDERING PHYSICIAN	SPECIMEN ID
NAME	MEDICAL FACILITY	SPECIMEN TYPE
DATE OF BIRTH	ADDITIONAL RECIPIENT	DATE OF COLLECTION
SEX	MEDICAL FACILITY ID	SPECIMEN RECEIVED
MEDICAL RECORD #	PATHOLOGIST	

Biomarker Findings

Blood Tumor Mutational Burden - 3 Muts/Mb
Microsatellite status - MSI-High Not Detected
Tumor Fraction - Elevated Tumor Fraction Not Detected

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

PIK3CA E545K, E726K
ERBB2 A775_G776insYVMA
NF1 G1092fs*14
DNMT3A splice site 1279+1G>A
ZNF217 amplification - equivocal†

† See About the Test in appendix for details.

Report Highlights

- Targeted therapies with **NCCN categories of evidence** in this tumor type: **Alpelisib + Fulvestrant** (p. 11), **Everolimus** (p. 15)
- Targeted therapies with **potential resistance** based on this patient's genomic findings: **Lapatinib** (p. 14)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 18)
- Variants that may represent **clonal hematopoiesis** and may originate from non-tumor sources: ***DNMT3A* splice site 1279+1G>A** (p. 8)

BIOMARKER FINDINGS

Blood Tumor Mutational Burden
- 3 Muts/Mb

Microsatellite status
- MSI-High Not Detected

Tumor Fraction
- Elevated Tumor Fraction Not Detected

GENOMIC FINDINGS		VAF %
PIK3CA -	E545K	3.1%
	E726K	2.5%

10 Trials see p. 22

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Biomarker Findings section

MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).

Tumor fraction is considered elevated when ctDNA levels are high enough that aneuploidy can be detected. The fact that elevated tumor fraction was not detected in this specimen indicates the possibility of lower levels of ctDNA but does not compromise confidence in any reported alterations. However, in the setting of a negative liquid biopsy result, orthogonal testing of a tissue specimen should be considered if clinically indicated (see Biomarker Findings section).

THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)		THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)	
Alpelisib + Fulvestrant	1	Everolimus	2A
		Temsirolimus	

Extensive evidence showing variant(s) in this sample may confer resistance to this therapy

NCCN category

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GENOMIC FINDINGS		VAF %	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
ERBB2 -	A775_G776insYVMA	1.4%	Ado-trastuzumab emtansine Fam-trastuzumab deruxtecan Trastuzumab Trastuzumab + Pertuzumab *Lapatinib* ⊗	None
10 Trials see p. 18				
NF1 -	G1092fs*14	2.5%	None	Selumetinib Trametinib
10 Trials see p. 20				

⊗ *Extensive evidence showing variant(s) in this sample may confer resistance to this therapy*

☐ *NCCN category*

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS (CH)

Genomic findings below may include nontumor somatic alterations, such as CH. The efficacy of targeting such nontumor somatic alterations is unknown. This content should be interpreted based on clinical context. Refer to appendix for additional information on CH.

DNMT3A - splice site 1279+1G>A ..... p. 8

GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

DNMT3A - splice site 1279+1G>A ..... p. 8

ZNF217 - amplification - equivocal ..... p. 9

NOTE Genomic alterations detected may be associated with activity of certain approved therapies; however, the therapies listed in this report may have varied clinical evidence in the patient's tumor type. Therapies and the clinical trials listed in this report may not be complete and/or exhaustive. Neither the therapies nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type. This report should be regarded and used as a supplementary source of information and not as the single basis for the making of a therapy decision. All treatment decisions remain the full and final responsibility of the treating physician and physicians should refer to approved prescribing information for all therapies. Therapies contained in this report may have been approved by the US FDA or other national authorities; however, they might not have been approved in your respective country. In the appropriate clinical context, germline testing of *APC*, *ATM*, *BAP1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FH*, *FLCN*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF1*, *NF2*, *PALB2*, *PMS2*, *POLE*, *PTEN*, *RAD51C*, *RAD51D*, *RB1*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *STK11*, *TGFBR2*, *TP53*, *TSC1*, *TSC2*, *VHL*, and *WT1* is recommended.

Variant Allele Frequency is not applicable for copy number alterations.

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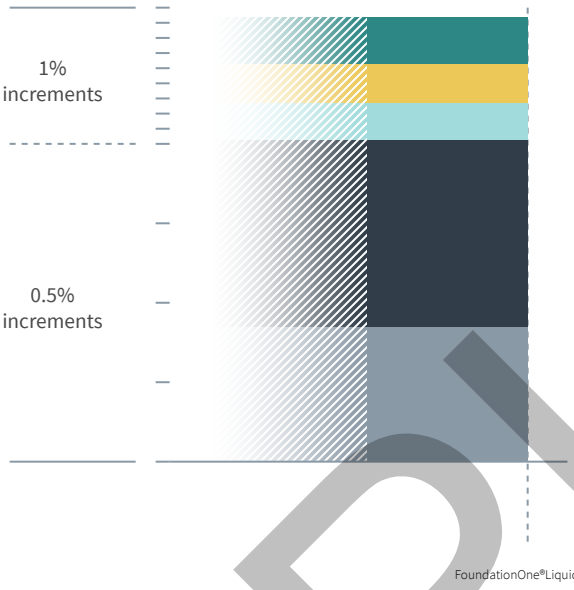

HISTORIC PATIENT FINDINGS		VAF%
Blood Tumor Mutational Burden		3 Muts/Mb
Microsatellite status		MSI-High Not Detected
Tumor Fraction		Elevated Tumor Fraction Not Detected
ERBB2	A775_G776insYVMA	1.4%
PIK3CA	E545K	3.1%
	E726K	2.5%
NF1	G1092fs*14	2.5%
DNMT3A	splice site 1279+1G>A	0.85%
ZNF217	amplification	Detected

NOTE This comparison table refers only to genes and biomarkers assayed by prior FoundationOne®Liquid CDx, FoundationOne®Liquid, FoundationOne®, or FoundationOne®CDx tests. Up to five previous tests may be shown.

For some genes in FoundationOne Liquid CDx, only select exons are assayed. Therefore, an alteration found by a previous test may not have been confirmed despite overlapping gene lists. Please refer to the Appendix for the complete list of genes and exons assayed. The gene and biomarker list will be updated periodically to reflect new knowledge about cancer biology.

As new scientific information becomes available, alterations that had previously been listed as Variants of Unknown Significance (VUS) may become reportable.

Tissue Tumor Mutational Burden (TMB) and blood TMB (bTMB) are estimated from the number of synonymous and non-synonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of ≥5%, and bTMB is calculated based on variants with an allele frequency of ≥0.5%.

Not Tested = not baited, not reported on test, or test preceded addition of biomarker or gene

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Not Detected = baited but not detected on test
Detected = present (VAF% is not applicable)
VAF% = variant allele frequency percentage
Cannot Be Determined = Sample is not of sufficient data quality to confidently determine biomarker status

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BIOMARKER

Blood Tumor Mutational Burden

RESULT
3 Muts/Mb

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in NSCLC and HSNCC, increased bTMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1[1-2] and anti-PD-1[3] therapies. In NSCLC, multiple clinical trials have shown patients with higher bTMB derive clinical benefit from immune checkpoint inhibitors following single agent or combination treatments with either CTLA4 inhibitors or chemotherapy, with reported high bTMB cutpoints ranging from 6 to 16 Muts/Mb[1]. In HNSCC, a Phase 3 trial showed that bTMB ≥16 Muts/Mb (approximate equivalency ≥8 Muts/Mb as measured by this assay) was associated with improved survival from treatment with a PD-L1 inhibitor alone or in combination with a CTLA-4 inhibitor[4].

FREQUENCY & PROGNOSIS

Average bTMB levels in solid tumors other than NSCLC have not been evaluated (cBioPortal, COSMIC, PubMed, Mar 2022)[5-7]. Published data investigating the prognostic implications of bTMB levels in breast cancer are limited (PubMed, Jul 2021). In a large study of patients with breast cancer, hypermutation was more frequently observed in metastatic tumors than in primary tumors[8]. In a study of 14,867 patients with breast cancer, high TMB was associated with older age and metastatic disease but was not significantly associated with PD-L1 positivity using the TMB cutoff of ≥10 Muts/Mb[9]. In estrogen receptor-positive breast cancer, increased TMB in tissue samples (>mean of 1.25 Muts/Mb) associated with shorter OS (HR=2.02) in an analysis of the TCGA data[10].

FINDING SUMMARY

Blood tumor mutational burden (bTMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations from circulating tumor DNA in blood. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma[11-12] and cigarette smoke in lung cancer[13-14], treatment with temozolomide-based chemotherapy in glioma[15-16], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[17-21], and microsatellite instability (MSI)[17,20-21]. High bTMB levels were not detected in this sample. It is unclear whether the bTMB levels in this sample would be predicted to be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents[1-3]. Depending on the clinical context, TMB testing of an alternate sample or by another methodology could be considered.

BIOMARKER

Tumor Fraction

RESULT
Elevated Tumor Fraction Not Detected

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Specimens with elevated tumor fraction values have high circulating-tumor DNA (ctDNA) content, and thus high sensitivity for identifying genomic alterations. Such specimens are at low risk of false negative results. However, if elevated tumor fraction is not detected, it does not exclude the presence of disease burden or compromise the confidence of reported alterations. Tumor fraction levels currently have limited implications for diagnosis, surveillance, or therapy and should not be overinterpreted or compared from one blood draw to another. There are currently no targeted approaches to address specific tumor fraction levels. In the research setting, changes in tumor fraction estimates have been associated with treatment duration and clinical response and may be a useful indicator for future cancer management[22-27].

FREQUENCY & PROGNOSIS

Detectible ctDNA levels have been reported in a variety of tumor types, with higher tumor fraction levels reported for patients with metastatic (Stage 4) tumors compared with patients with localized disease (Stages 1 to 3)[28]. Elevated tumor fraction levels have been reported to be associated with worse prognosis in a variety of cancer types, including pancreatic cancer[29], Ewing sarcoma and osteosarcoma[30], prostate cancer[25], breast cancer[31], leiomyosarcoma[32], esophageal cancer[33], colorectal cancer[34], and gastrointestinal cancer[35].

FINDING SUMMARY

Tumor fraction provides an estimate of the percentage of ctDNA present in a cell-free DNA (cfDNA) sample. The tumor fraction estimate for this sample is based on the observed level of aneuploid instability. The tumor fraction algorithm utilized for FoundationOne Liquid CDx uses the allele frequencies of approximately 1,000 single-nucleotide polymorphism (SNP) sites across the genome. Unlike the maximum somatic allele frequency (MSAF) method of estimating ctDNA content[36], the tumor fraction metric does not take into account the allele frequency of individual variants but rather produces a more holistic estimate of ctDNA content using data from across the genome. The amount of ctDNA detected may correlate with disease burden and response to therapy[37-38].

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GENE
ERBB2

ALTERATION
A775_G776insYVMA

TRANSCRIPT ID
NM_004448

CODING SEQUENCE EFFECT
2324_2325insATACGTGATGGC

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of extensive clinical evidence, ERBB2 amplification or activating mutation may predict sensitivity to therapies targeting HER2, including antibodies such as trastuzumab[39-44], pertuzumab in combination with trastuzumab[41,45-47], and zanidatamab (ZW25)[48], as well as antibody-directed conjugates such as ado-trastuzumab emtansine (T-DM1)[49] and fam-trastuzumab deruxtecan[50], HER2 kinase inhibitors such as tucatinib[51-54], and dual EGFR/HER2 kinase inhibitors such as lapatinib[55-63], afatinib[44,64-73], neratinib[74-77], dacomitinib[78], and pyrotinib[79-80] . Patients with ERBB2-mutated breast cancer have benefited from HER2-targeted therapies. In patients with HR+ breast cancer, the triple combination of neratinib plus trastuzumab and fulvestrant achieved an ORR of 42% (14/33, 1CR) and the combination of neratinib plus fulvestrant elicited an ORR of 29% (4/14)[81]. For patients with triple negative breast cancer (TNBC), the combination of neratinib plus trastuzumab achieved an ORR of 33% (6/18, 1 CR)[81]. Pyrotinib has demonstrated an ORR of 40% (4/10) for patients with ERBB2-mutated breast cancer that was not HER2-amplified[82]. Individual patients have benefited from other HER2-targeted regimens including the triple combination of trastuzumab, pertuzumab and fulvestrant[41] and lapatinib plus trastuzumab[59,83].

— Potential Resistance —

Clinical and preclinical data suggest that ERBB2 exon 20 insertions confer resistance to lapatinib and reduced sensitivity to afatinib, dacomitinib, and neratinib[44,70-71,76,78,84-89]. However, it is unclear if ERBB2 exon 20 insertions confer reduced sensitivity to lapatinib in combination with other therapies, such as trastuzumab. For patients with breast cancer, retrospective[90-93] and Phase 2[94] studies have reported that concurrent PIK3CA or PTEN alterations that activate the PI3K pathway are associated with resistance to therapies that target HER2, including trastuzumab and lapatinib, although other retrospective[95] and Phase 2[96] studies have reported conflicting results.

FREQUENCY & PROGNOSIS

ERBB2 exon 20 mutations have been reported in 0.4–0.9% of breast cancer cases, with the most common of these being A775_G776insYVMA (32-46%) and G778_P780insGSP (46-64%)[87,97]. In the TCGA dataset, ERBB2 amplification was detected in 13% of breast invasive carcinoma cases[98]. ERBB2 mutations have been reported in 1-3% of breast invasive carcinoma cases[97-99]. The incidence of ERBB2 alterations has been found to be significantly enriched in CDH1-mutated invasive lobular breast cancers[100]. HER2 is predicted to be overexpressed (as assessed by FISH, CNV analysis, or immunohistochemistry) in 12-25% of breast cancers[101-103]. Phosphorylated HER2 was expressed in 62.5% (55/88) of HER2-positive breast cancers[104]. For patients with breast cancer and positive axillary lymph nodes, amplification of HER2 was correlated with shorter time to relapse and overall survival as compared with patients with non-amplified tumors by univariate and multivariate analysis, with greater differences observed in patients whose tumors harbored >5 copies of HER2[105]. Retrospective analysis has reported that patients with low-grade, node-negative, HER2-positive breast cancer have a 5-year survival rate of 68% compared with 96% for patients with HER2-negative tumors[106]. Alterations in ZNF703, ERBB2, MDM2, PALB2, ARFRP1, IRS2, and JAK2 may be associated with resistance to CDK4/6 inhibitors and impaired PFS for patients with HR+ metastatic breast cancer, according to a retroactive study of 131 patients[107]. Acquisition of resistance to trastuzumab was correlated with negativity for pHER2 (p=0.028) for patients with HER2-positive breast cancer[104].

FINDING SUMMARY

ERBB2 (also known as HER2) encodes a receptor tyrosine kinase which is in the same family as EGFR. ERBB2 exon 20 insertion mutations, such as observed here, are predicted to be activating[86-87,108-110]. The mutation seen here is similar to A775_G776insYVMA (also known as A771_Y772insYVMA or Y772_A775dup), which is the most common exon 20 insertion mutation across cancer types[87].

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GENE
PIK3CA

ALTERATION
E545K, E726K

TRANSCRIPT ID
NM_006218, NM_006218

CODING SEQUENCE EFFECT
1633G>A, 2176G>A

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Clinical and preclinical data in various tumor types indicate that PIK3CA activating alterations may predict sensitivity to therapies targeting PI3K[111-118], AKT[119-120], or mTOR[121-128]. In the Phase 3 SOLAR-1 study, the addition of alpelisib to fulvestrant statistically improved PFS (11.0 vs. 5.7 months, HR=0.65) and ORR (27% vs. 13%) and numerically improved median OS (39.3 vs. 31.4 months, HR=0.86) in PIK3CA-mutated hormone receptor-positive (HR+), HER2-negative breast cancer compared with placebo with fulvestrant, but not in PIK3CA-wildtype HR+, HER2-negative breast cancer[129]. In a Phase 2 trial, the addition of the AKT inhibitor capivasertib to fulvestrant resulted in a numerically increased median PFS (mPFS) for patients with PIK3CA- or PTEN-altered, HR+, HER2-negative metastatic breast cancer (9.5 vs. 5.2 months)[130]. Single-agent capivasertib also demonstrated activity in a Phase 1 study[131]. In trials of AKT inhibitors with paclitaxel, neither capivasertib nor ipatasertib showed significant mPFS benefit for patients with PI3K pathway-mutated, HR+, HER2-negative metastatic breast cancer compared with paclitaxel plus placebo[132]. In a Phase 1 study, the PIK3CA-selective inhibitor inavolisib (GDC-0077) alone or in combination with endocrine therapy (letrozole or fulvestrant) with or without palbociclib yielded an ORR of 32% (23/73) for patients with PIK3CA-mutated HR+, HER2-negative breast cancer, with an ORR of 40% (6/15) observed for patients who received inavolisib plus palbociclib and fulvestrant[133-134] . A Phase 1 study of combination palbociclib, fulvestrant, and the pan-PIK3CA inhibitor taselisib reported an ORR of 38% (9/24), DCR of 58% (14/24), and mPFS of 7.2 months for patients with PIK3CA-mutated ER+, HER2-negative breast cancer[135]. The addition of the MTOR inhibitor everolimus to exemestane to treat HR+, HER2-negative advanced breast cancer has shown clinical benefit, regardless of PIK3CA status[136-137]. Phase 3 trials of the pan-PIK3CA inhibitors buparlisib or taselisib to fulvestrant demonstrated activity for patients with PIK3CA-mutated HR+ breast cancer but were discontinued due to insufficient efficacy (buparlisib)[138] or unacceptable adverse event frequency (taselisib)[139]. A Phase 2 trial of capivasertib with paclitaxel versus paclitaxel alone showed a median OS benefit (19.1 vs. 13.5 months) both for patients with AKT1, PTEN, or PIK3CA-mutated triple-negative breast cancer (TNBC; HR=0.58, 95% CI 0.2-1.6) and for patients with TNBC without PI3K-pathway mutations (HR=0.74, 95% CI 0.47-1.18)[140]. Despite promising initial results in earlier trials, the Phase 3 IPATunity130 trial for patients with AKT1, PTEN, or PIK3CA-mutated TNBC failed to show improved PFS for first-line ipatasertib in combination with paclitaxel relative to paclitaxel alone (7.4 vs. 6.1 months)[141]. The Phase 2 NCI-MATCH study of copanlisib for patients with refractory solid tumors harboring PIK3CA mutations with or without PTEN loss met its primary endpoint with an ORR of 16% (4/25 PRs); responses (PR or SD >6 months) were seen in patients with ameloblastoma, liposarcoma, and carcinomas of the endometrium, ovary, esophagus, lung, and prostate[118]. However, the Phase 2 study of copanlisib for patients with endometrial carcinoma harboring PIK3CA hotspot mutations failed to report any objective responses (n=11)[117]. Two other studies of copanlisib for patients with genomically unselected tumors reported 1 CR and 2 PRs (1 unconfirmed) among 16 total patients with PIK3CA-mutated solid tumors with or without PTEN alterations[115-116].

— Potential Resistance —

For patients with breast cancer, retrospective[90-93] and Phase 2[94] studies have reported that concurrent PIK3CA or PTEN alterations that activate the PI3K pathway are associated with resistance to therapies that target HER2, including trastuzumab and lapatinib, although other retrospective[95] and Phase 2[96] studies have reported conflicting results.

FREQUENCY & PROGNOSIS

Mutations in PIK3CA have been reported in 25-40% of breast cancer cases[98,142-146]. In the randomized Phase 2 SAFIR02 trial, PIK3CA mutations were associated with reduced OS in patients with hormone-receptor-positive (HR+)/HER2 negative (HER-) metastatic breast cancer but with improved OS in patients with mTNBC compared to patients with PIK3CA wildtype status[146]. Although double PIK3CA mutations were frequently observed in HR+/HER2- breast cancers, as compared with other receptor subtypes (15% vs. 5.4%, p=0.004), this did not impact invasive disease-free survival or OS for patients when compared with single PIK3CA mutations by univariate and multivariate analysis in 1 retrospective study[147]. For patients with HER2+ breast cancer receiving trastuzumab and pertuzumab with chemotherapy, PIK3CA mutations significantly associated with shorter PFS (13 vs. 23 months; HR=1.98)[148]. Mutations in coding exon 20 (H1047R) of PIK3CA have been associated with a better prognosis in breast carcinoma than mutations occurring in coding exon 9 (E542K)[149].

FINDING SUMMARY

PIK3CA encodes p110-alpha, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates a number of critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival[150-151]. PIK3CA alterations that have been characterized as activating, such as observed here, are predicted to be oncogenic[152-173].

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GENE
NF1

ALTERATION
G1092fs*14

TRANSCRIPT ID
NM_001042492

CODING SEQUENCE EFFECT
3273_3274insT

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in neurofibromatosis Type 1-associated neurofibroma[174-177], glioma or glioblastoma[177-181], and non-small cell lung cancer[182], NF1 inactivation may predict sensitivity to MEK inhibitors such as cobimetinib, trametinib, binimetinib, and selumetinib. Loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors, including everolimus and temsirolimus, based on limited clinical data[122,183-184] and strong preclinical data in models of malignant peripheral nerve sheath tumor (MPNST)[185-186]. A preclinical study suggests that combined mTOR and MEK inhibition is effective in a model of NF1-deficient MPNST[187]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[188], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[189].

FREQUENCY & PROGNOSIS

Two large genomic studies for patients with breast cancer in the metastatic setting reported NF1 mutation or deletion in 5.2-11% of ER+/HER2-, 9.1-11% of HER2+, and 6.2-8.6% of TNBC cases[190-191]. NF1 alterations are enriched in metastatic breast invasive lobular carcinoma (ILC) compared to metastatic invasive ductal carcinoma (12.2% vs 3.1%), and are often mutually exclusive with ESR1 alterations[192]. NF1 alterations have been reported to arise during endocrine therapy resistance in ILC[192]. Studies have suggested that women with neurofibromatosis type 1, which is associated with germline NF1 mutations, may have an increased risk of breast cancer[193-197]. Published data investigating the prognostic implications of NF1 alteration in breast cancer are limited (PubMed, Feb 2022).

FINDING SUMMARY

NF1 encodes neurofibromin, a GTPase-activating protein (GAP) that is a key negative regulator of the RAS signaling pathway[198]. Neurofibromin acts as a tumor suppressor by repressing RAS signaling[199]. Alterations such as seen here may disrupt NF1 function or expression[199-208].

POTENTIAL GERMLINE IMPLICATIONS

Germline mutations in NF1 cause the autosomal dominant disorder neurofibromatosis type 1, which is characterized in part by increased risk of developing various tumors, including sarcoma, glioma, breast carcinoma, and neuroendocrine and hematological neoplasms[197,209-210]. Estimates for the prevalence of the disorder in the general population range from 1:2,500 to 1:3,000[211-212], and in the appropriate clinical context, germline testing of NF1 is recommended.

GENE
DNMT3A

ALTERATION
splice site 1279+1G>A

TRANSCRIPT ID
NM_022552

CODING SEQUENCE EFFECT
1279+1G>A

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no targeted therapies available to address genomic alterations in DNMT3A in solid tumors.

FREQUENCY & PROGNOSIS

DNMT3A alterations have been reported at relatively low frequencies in solid tumors and are more prevalent in hematological malignancies (cBioPortal, Feb 2022)[5-6]. Published data investigating the prognostic implications of DNMT3A alterations in solid tumors are limited (PubMed, Feb 2022).

FINDING SUMMARY

The DNMT3A gene encodes the protein DNA methyltransferase 3A, an enzyme that is involved in the methylation of newly synthesized DNA, a function critical for gene regulation[213-214]. The role of DNMT3A in cancer is uncertain, as some reports describe increased expression and contribution to tumor growth, whereas others propose a role for DNMT3A as a tumor suppressor[215-220]. Alterations such as seen here may disrupt DNMT3A function or expression[221-224].

POTENTIAL CLONAL HEMATOPOIESIS IMPLICATIONS

Variants seen in this gene have been reported to occur in clonal hematopoiesis (CH), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion[225-230]. CH in this gene has been associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy[225-226]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[231]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[229,232-233]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

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GENE
ZNF217

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no available targeted therapies to address genomic alterations in ZNF217. Expression of ZNF217 may predict relapse of estrogen receptor (ER)-positive breast cancer under hormone therapy through its direct interaction with ER-alpha[234-235]. ZNF217 overexpression has also been associated with resistance to paclitaxel[236] and doxorubicin[237] in breast cancer cell lines. ZNF217 has been suggested as a potential biomarker for treatment with the DNA synthesis inhibitor and AKT inhibitor triciribine in breast cancer based on preclinical findings in cultured cells and xenografts expressing high levels of ZNF217; triciribine treatment also restored sensitivity to doxorubicin in these cells[238].

FREQUENCY & PROGNOSIS

Amplification and/or overexpression of ZNF217 has been reported in breast[239], ovarian[240-241], gastric[242-243], colon[244], prostate[245], esophageal[246], and urothelial carcinomas[247], glioblastoma[248], and ovarian carcinosarcomas[249]. Overexpression in these tumors has generally been linked with aggressive tumor behavior and poor clinical prognosis. High levels of ZNF217 expression result in dysregulation of a broad range of genes that may contribute to tumorigenesis[250-252], and increased expression or activation of ERBB3[239,253], FAK[239], Aurora kinase A[236], AKT[237], and TGF-beta/SMAD signaling[239] has been demonstrated in ZNF217-expressing tumors or cells.

FINDING SUMMARY

ZNF217 encodes a candidate oncogene that has likely roles in histone modification and transcriptional repression[237,254]. ZNF217 amplification has been correlated with protein overexpression in breast carcinoma tumors and cell lines[255]. The role of ZNF217 in promoting tumorigenesis was established in preclinical studies demonstrating that expression of ZNF217 results in the immortalization of both human mammary epithelial cells and ovarian surface epithelial cells in culture[256-257].

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Ado-trastuzumab emtansine

Assay findings association

ERBB2
A775_G776insYVMA

AREAS OF THERAPEUTIC USE

Ado-trastuzumab emtansine (T-DM1) is an antibody-drug conjugate that targets the protein ERBB2/HER2 on the cell surface, which inhibits HER2 signaling; it also releases the cytotoxic therapy DM1 into cells, leading to cell death. T-DM1 is FDA approved to treat patients with HER2-positive (HER2+) metastatic breast cancer and disease progression on prior therapy as well as patients with HER2+ early breast cancer who have residual invasive disease after neoadjuvant taxane and trastuzumab-based treatment. Please see the drug label for full prescribing information.

GENE ASSOCIATION

ERBB2 amplification or activating mutations may predict sensitivity to T-DM1[49,258-273] . Patients with NSCLC and various ERBB2 exon 20 insertion mutations have benefited from T-DM1[261,274-275] .

SUPPORTING DATA

For patients with HER2-positive breast cancer (BC) previously treated with HER2-directed therapies, Phase 3 trials of single-agent ado-trastuzumab emtansine (T-DM1) have reported significant increases in median PFS (mPFS) compared with the physician's choice of therapy (6.2 vs. 3.3 months)[262] or lapatinib plus capecitabine (9.6 vs. 6.4 months)[49,263,267] . The Phase 3 DESTINY-Breast03 study for patients with HER2-positive metastatic BC (mBC) previously treated with trastuzumab and taxane reported significantly improved mPFS for patients treated with fam-trastuzumab deruxtecan (T-DXd) compared with T-DM1 (not reached vs. 6.8 months, HR=0.28)[276]. The Phase 3 MARIANNE study for patients with HER2-positive advanced BC treated in the first line with T-DM1 reported no significant differences in ORR (60%, 64%, and 68%) or mPFS (14.1, 15.2, and 13.7 months) when comparing T-DM1 combined with placebo, T-DM1 with pertuzumab, and trastuzumab with taxane, respectively[268]; however, an earlier Phase 2 study reported improved mPFS with T-DM1 compared with trastuzumab plus docetaxel (14.2 months vs. 9.2 months, HR=0.59) in this setting[269]. In the Phase 3 KATHERINE study, patients with HER2-positive early BC with residual invasive disease following completion of neoadjuvant taxane and trastuzumab treated with T-DM1 experienced significantly higher invasive disease-free survival rates at 3 years (88% vs. 77%, HR=0.50) compared with patients treated with trastuzumab[270]. In the neoadjuvant setting, the Phase 3 KRISTINE study for patients with HER2-positive BC reported a lower pathologic CR rate (44% vs. 56%, p=0.016) with T-DM1 plus pertuzumab compared with the combination of trastuzumab, pertuzumab, docetaxel, and carboplatin[271]. Patients with HER2-positive locally advanced BC or mBC have experienced clinical benefit in Phase 1/2 studies from T-DM1 in combination with docetaxel[272], paclitaxel with or without pertuzumab (Krop et al., 2016;), neratinib[277], alpelisib[278], and tucatinib[277]. A retrospective analysis found that patients with HER2-positive mBC and active central nervous system (CNS) metastases treated with T-DM1 achieved an ORR of 40% (4/10); there was no significant OS difference between patients with and without CNS metastases[279].

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Alpelisib + Fulvestrant

Assay findings association

PIK3CA
E545K, E726K

AREAS OF THERAPEUTIC USE

Alpelisib is a phosphatidylinositol 3-kinase (PI3K) inhibitor with selective activity against the alpha isoform (PI3K-alpha), and fulvestrant is an estrogen receptor (ER) antagonist and selective estrogen receptor degrader (SERD). The combination is FDA approved to treat men and postmenopausal women with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated advanced breast cancer. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of prospective clinical data, PIK3CA mutations including C420R, E542K, E545A, E545G, E545K, E545D, Q546E, Q546R, H1047L, H1047Y, and H1047R are associated with sensitivity to alpelisib in combination with fulvestrant. In ER+/HER2– breast cancer, PFS benefit from the addition of alpelisib to fulvestrant was specifically observed for patients with PIK3CA mutations (11.0 vs. 5.7 months, HR=0.65), including patients with PIK3CA exon 9 or exon 20 mutations[119].

SUPPORTING DATA

In the Phase 3 SOLAR-1 study for patients with HR+/HER2- endocrine therapy-resistant advanced breast cancer, the addition of alpelisib to fulvestrant significantly improved median PFS (mPFS; 11.0 vs. 5.7 months, HR=0.65), ORR (27% vs. 13%), and clinical benefit rate (62% vs. 45%), and numerically improved median OS (mOS; 39.3 vs. 31.4 months, HR=0.86) for patients with PIK3CA mutations[119,280] . Benefit was observed for patients with PIK3CA exon 9 or exon 20 mutations[119]; for patients with wildtype PIK3CA, the addition of alpelisib to fulvestrant did not significantly improve mPFS (7.4 vs. 5.6 months, HR=0.85)[119]. This trial excluded patients with active brain metastases; however, control of progressive brain metastases (1/4 PR and 2/4 SDs by response assessment in neuro-oncology brain metastases criteria) was reported in a case series of 4 patients with PIK3CA-mutated HR+/HER2- breast cancer treated with alpelisib in combination with either fulvestrant or exemestane[281]. The Phase 2 BYLieve study for previously treated patients with PIK3CA-mutated HR+/HER2- advanced breast cancer reported an ORR of 19%, mPFS of 7.3 months, and mOS of 26.4 months for patients treated with alpelisib plus fulvestrant following progression on a CDK4/6 inhibitor in combination with an aromatase inhibitor[282]; an ORR of 16% and mPFS of 5.7 months for patients treated with alpelisib plus letrozole following progression on a CDK4/6 inhibitor in combination with fulvestrant[283]; and an ORR of 24% and mPFS of 5.6 months for patients treated with alpelisib plus fulvestrant who had previously progressed on aromatase inhibitors and received chemotherapy or endocrine therapy[284]. Biomarker analysis of the BYLieve trial reported that alpelisib combination regimens were effective for patients previously treated with CDK4/6 inhibitors independent of their tumor genomic profile (including tumor mutational burden [TMB] status and the presence of genes associated with CDK4/6 inhibitor resistance)[284-285] , though concurrent ESR1 mutations were associated with significantly shorter mPFS for patients treated with alpelisib plus letrozole (HR=0.55)[286].

Fam-trastuzumab deruxtecan

Assay findings association

ERBB2
A775_G776insYVMA

AREAS OF THERAPEUTIC USE

Fam-trastuzumab deruxtecan is an antibody-drug conjugate that targets the protein ERBB2/HER2 on the cell surface and delivers the cytotoxic payload DXd, which inhibits DNA topoisomerase I to induce DNA damage. Fam-trastuzumab deruxtecan is FDA approved to treat patients with HER2-positive breast cancer and gastric or gastroesophageal junction adenocarcinoma who have received prior HER2-targeted therapy. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data in non-small cell lung cancer (NSCLC)[287-288] , ERBB2 missense or exon 20 insertion mutations may predict sensitivity to fam-trastuzumab deruxtecan.

SUPPORTING DATA

The Phase 3 DESTINY-Breast03 study for patients with HER2-positive metastatic breast cancer (mBC) previously treated with trastuzumab and taxane reported a significantly improved median PFS (mPFS) for patients treated with fam-trastuzumab deruxtecan (T-DXd) compared with ado-trastuzumab emtansine (T-DM1) (not reached vs. 6.8 months, HR=0.28)[276,289] . The Phase 2 DESTINY-Breast01 study of T-DXd for patients with HER2-positive mBC previously treated with T-DM1 reported a 61% ORR (6.0% CR) and a 97% DCR with a mPFS of 16.4 months[50]. A Phase 1 trial reported similar results (60% ORR, 94% DCR, mPFS of 22.1 months) for patients with pre-treated ERBB2-positive breast cancer[290]. A Phase 1b study evaluating T-DXd to treat patients with heavily pre-treated breast cancer expressing low levels of ERBB2 reported an ORR of 37% (20/54) and a median duration of response of 10.4 months[291].

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Trastuzumab

Assay findings association

ERBB2
A775_G776insYVMA

AREAS OF THERAPEUTIC USE

Trastuzumab is a monoclonal antibody that targets the protein ERBB2/HER2. It is FDA approved as monotherapy and in combination with chemotherapy for HER2+ metastatic gastric or gastroesophageal adenocarcinoma. Trastuzumab biosimilars are also FDA approved for these indications. Please see the drug label(s) for full prescribing information.

GENE ASSOCIATION

Trastuzumab-involving regimens elicited significant responses in patients with certain ERBB2 mutations[43-44,59,84,292-293] . Patients with NSCLC and ERBB2 exon 20 insertions, including A775_G776insYVMA and G776>VC, have benefited from treatment with trastuzumab[43-44,84,293-294] , with reported DCRs of 75–96% for trastuzumab in combination with chemotherapy[44,84] .

SUPPORTING DATA

A Phase 3 study of adjuvant trastuzumab with chemotherapy for patients with metastatic HER2-positive breast cancer (HER2+ BC) demonstrated significant improvements in OS, time to progression, and ORR[39]. Trastuzumab biosimilars demonstrated comparable clinical benefit to trastuzumab for patients with HER2+ BC[295-303] . In the Phase 3 NOAH study for patients with HER2+ BC, neoadjuvant trastuzumab plus chemotherapy resulted in improved 5-year event-free survival (EFS) compared with neoadjuvant chemotherapy alone (58% vs. 43%)[304]. The Phase 3 CLEOPATRA study of first-line trastuzumab with pertuzumab and docetaxel for patients with metastatic HER2+ BC reported significantly improved median PFS (18.7 vs. 12.4 months, HR=0.69) and median OS (57.1 vs. 40.8 months, HR=0.69) compared with trastuzumab plus docetaxel[45-46,305-306] . The Phase 3 NeoALTTO trial for patients with early-stage HER2+ BC treated with lapatinib, trastuzumab, or a combination of both reported 3-year EFS rates of 78%, 76%, and 84%, and 3-year OS rates of 93%, 90%, and 95%, respectively[307]. Two Phase 3 studies comparing 6-month with 12-month adjuvant trastuzumab reported similar disease-free survival (DFS) rates for patients with HER2+ early-stage BC after 5.4 years (89.4% vs. 89.8%, HR=1.07)[308] or 7.5-year median follow-up (78.8% vs. 79.6%, HR=1.08)[309]. The randomized Phase 3 NSABP B-47 study reported that the addition of trastuzumab to adjuvant chemotherapy did not significantly improve invasive disease-free survival (IDFS) for patients with HER2-low BC (defined as IHC score of 1+ or 2+ in the absence of gene amplification) compared with chemotherapy alone (5-year IDFS rates of 89.8% vs. 89.2%, HR=0.98; p=0.85); this response was reported regardless of lymph node involvement or HR status[310]. A Phase 2 analysis reported 5-year distant DFS rates of 92% for patients with HER2+ early-stage BC treated with chemotherapy and trastuzumab, and 89% for patients treated with lapatinib and chemotherapy[311]. In the Phase 3 BOLERO-1 trial, first-line treatment with everolimus and trastuzumab plus paclitaxel versus placebo for patients with HER2+ advanced BC did not significantly improve median PFS (15.0 vs. 14.5 months); however, the regimen increased PFS in the HR-negative subpopulation (20.3 vs. 13.1 months, HR=0.66)[312]. Everolimus plus trastuzumab with vinorelbine prolonged median PFS (7.0 vs. 5.8 months, HR=0.78), relative to the addition of placebo, for patients with trastuzumab-resistant HER2+ BC treated in the Phase 3 BOLERO-3 trial[313]. In a Phase 2 trial for patients with HER2+ metastatic BC previously treated with HER2-targeting agents, tucatinib plus trastuzumab and capecitabine significantly extended median PFS (7.8 vs. 5.6 months) and increased the 1-year median PFS rate (33.1% vs. 12.3%, HR=0.54) and 2-year median OS rate (44.9% vs. 26.6%, HR=0.66) compared with placebo with trastuzumab and capecitabine[51]. For patients with HR+, HER2+ BC who had received prior HER2-targeted therapy, abemaciclib combined with trastuzumab and fulvestrant compared with abemaciclib plus trastuzumab or trastuzumab plus chemotherapy significantly improved median PFS (8.3 vs. 5.7 vs. 5.7 months) and ORR (35.7% vs. 16.2% vs. 15.9%) in Phase 2 monarcHER study[314].

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Trastuzumab + Pertuzumab

Assay findings association

ERBB2
A775_G776insYVMA

AREAS OF THERAPEUTIC USE

Trastuzumab is a monoclonal antibody that targets ERBB2/HER2, and pertuzumab is a monoclonal antibody that interferes with the interaction between HER2 and ERBB3. These therapies are FDA approved in combination for the treatment of patients with HER2-positive (HER2+) metastatic breast cancer who have not received prior chemotherapy or HER2-targeted therapy. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical studies in multiple tumor types, ERBB2 amplification or activating mutations may predict sensitivity to trastuzumab in combination with pertuzumab[46,315-320] .

SUPPORTING DATA

For patients with HER2+ breast cancer receiving trastuzumab and pertuzumab with chemotherapy, ERBB2 amplification detected by NGS significantly associated with improved PFS (22.8 vs. 9.4 months; HR=1.79)[148]. In a case report, a patient with breast cancer and ERBB2 S310F had 12 months of clinical benefit from the combination of trastuzumab, pertuzumab, and fulvestrant[41]. The CLEOPATRA Phase 3 randomized trial for patients with HER2+ metastatic breast cancer (MBC) reported that the addition of pertuzumab to first-line trastuzumab and docetaxel demonstrated a significant improvement in median PFS (mPFS; 18.7 vs. 12.4 months, HR=0.69) and median OS (mOS; 57.1 vs. 40.8 months, HR=0.69) compared with the addition of placebo to this regimen[45-46,305-306] . Superior clinical benefit has been observed in multiple clinical studies in which pertuzumab was added to the combination of trastuzumab plus chemotherapy, as compared with other combinations of pertuzumab, trastuzumab, and/or chemotherapy, for patients with HER2+ MBC and locally advanced breast cancer (LABC)[319,321-324] . For patients with HER2+ and hormone receptor-positive (HR+) MBC/LABC, addition of pertuzumab to trastuzumab plus an aromatase inhibitor (AI) significantly increased mPFS compared with trastuzumab plus AI alone (20.6 vs. 15.8 months, respectively; HR=0.67) but did not significantly improve mOS (60.2 vs. 57.2 months, respectively; HR=1.05)[325]. In the Phase 3 APHINITY study for patients with HER2+ early-stage breast cancer, the addition of pertuzumab to chemotherapy plus trastuzumab as adjuvant treatment improved the estimated 3-year rate of invasive disease-free survival (IDFS) compared with the addition of placebo to this regimen (94% vs. 93%), with greater improvement seen for patients with node-positive (92% vs. 90%, HR=0.77) versus node-negative (97.5% vs. 98.4%, HR=1.13) disease[316]. Clinical benefit for HER2+ early-stage breast cancer was also reported for patients treated with pertuzumab, trastuzumab, and chemotherapy in the neoadjuvant setting followed by pertuzumab combined with trastuzumab in the adjuvant setting[326]. In the Phase 3 KRISTINE trial, patients with HER2+ Stage 2 to Stage 3 breast cancer treated in the neoadjuvant setting experienced an increased number of pathological CRs (pCRs) when treated with pertuzumab, trastuzumab, and chemotherapy, compared with those treated with trastuzumab emtansine plus pertuzumab (56% vs. 44%, respectively)[315].

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Lapatinib

Resistance of variant(s) to associated therapy is likely

Assay findings association

ERBB2
A775_G776insYVMA

AREAS OF THERAPEUTIC USE

Lapatinib is a tyrosine kinase inhibitor that targets EGFR, ERBB2/HER2, and to a lesser degree, ERBB4. It is FDA approved in combination with capecitabine to treat patients with HER2-overexpressing (HER2+) metastatic breast cancer. Please see the drug label for full prescribing information.

GENE ASSOCIATION

Activation or amplification of ERBB2 may predict sensitivity to lapatinib[55-63] . On the basis of clinical and preclinical evidence, ERBB2 exon 20 insertions confer resistance to lapatinib[44,84-88] . However, it is unclear if ERBB2 exon 20 insertions confer reduced sensitivity to lapatinib in combination with other therapies, such as trastuzumab.

SUPPORTING DATA

Lapatinib as a treatment for HER2+ breast cancer has primarily been investigated in combination with other chemotherapeutic agents; these combination regimens have been shown to extend PFS as well as to extend OS in some instances[56-57,327-330] . However, multiple Phase 3 trials have shown superior clinical outcomes to lapatinib plus capecitabine with other HER2-targeted agents in certain settings, including trastuzumab plus taxane as first-line therapy for HER2+ metastatic breast cancer[331] and ado-trastuzumab emtansine (T-DM1) for patients who have progressed on trastuzumab plus taxane[49]. Phase 3 studies of adjuvant lapatinib have reported no significant disease-free survival benefit compared with placebo[332] or trastuzumab[333]. Phase 2/3 trials in the neoadjuvant setting have found that the combination of lapatinib and trastuzumab may result in numerically improved ORRs compared with either drug alone[307,328-329] .

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Everolimus

Assay findings association

PIK3CA
E545K, E726K

AREAS OF THERAPEUTIC USE

Everolimus is an orally available mTOR inhibitor that is FDA approved to treat renal cell carcinoma (RCC) following antiangiogenic therapy; pancreatic neuroendocrine tumors; and well-differentiated non-functional neuroendocrine tumors of the lung or gastrointestinal tract. Everolimus is also approved to treat either renal angiomyolipoma or subependymal giant cell astrocytoma in association with tuberous sclerosis complex (TSC). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence[121-128] , PIK3CA activating mutations may predict sensitivity to mTOR inhibitors such as everolimus and temsirolimus. Studies have reported modest activity of these therapies as single agents (ORRs of 0-4%), but improved activity has been observed when they are combined with other agents such as bevacizumab and doxorubicin (ORRs of 25-44%), for patients with PIK3CA-mutated solid tumors[125-128,334-338] .

SUPPORTING DATA

Clinical benefit has been reported for patients with PIK3CA-mutated breast cancer treated with everolimus as a single agent[339] or in combination with gemcitabine and cisplatin[340]. In the Phase 3 BOLERO-2 study for hormone receptor-positive (HR+), HER2-negative (HER2–) breast cancer, the addition of everolimus to exemestane improved median PFS (mPFS) in both the first-line exploratory cohort (11.5 vs. 4.1 months, HR=0.39)[341] and second-line cohort (7.8 vs. 3.2 months, HR=0.45)[342-344] compared with exemestane alone. Combination everolimus and exemestane modestly improved mPFS compared with everolimus alone in the BOLERO-6 randomized clinical trial (8.4 vs. 6.8 months, HR=0.74)[345]. Patients with HR+, HER2– breast cancer also benefited from everolimus combined with other anti-estrogen therapies, including letrozole, tamoxifen, and anastrozole[346-348] . For patients with HR+, HER2– breast cancer who progressed on anti-estrogen therapies, addition of everolimus to the most recent endocrine therapy elicited mPFS of 6.6 months[349]. For patients with HER2+ breast cancer, everolimus combined with trastuzumab plus paclitaxel did not significantly improve mPFS in the full study population (15.0 months with everolimus vs. 14.5 months with placebo), but increased PFS in the HR-negative subpopulation (20.3 vs. 13.1 months)[312]. For patients with trastuzumab-resistant HER2+ breast cancer, the addition of everolimus to trastuzumab plus vinorelbine prolonged mPFS (7.0 vs. 5.8 months)[313], whereas for HER2– breast cancer, addition of everolimus to vinorelbine in the second-line did not improve mPFS (4.0 vs. 4.1 months)[350]. In the Phase 3 UNIRAD study for patients with high-risk early HR+/HER2– breast cancer, the addition of everolimus to adjuvant hormone therapy did not improve 3-year disease-free survival (HR=0.95), metastasis-free survival (HR=0.88), or OS (HR=1.09) relative to hormone therapy plus placebo[351]. Patients with metastatic triple-negative breast cancer treated with everolimus plus carboplatin achieved a clinical benefit rate of 36% (9/25)[352]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[188], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[189].

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Selumetinib

Assay findings association

NF1
G1092fs*14

AREAS OF THERAPEUTIC USE

Selumetinib is a MEK inhibitor that is FDA approved to treat pediatric patients 2 years of age and older with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas (PNs). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence in neurofibromatosis type 1 (NF1)-associated neurofibroma[174-177,353-357] , glioma[177-181,358] , and non-small cell lung cancer[182], NF1 inactivation may predict sensitivity to MEK inhibitors.

SUPPORTING DATA

In a Phase 2 study for post-menopausal patients with endocrine sensitive breast cancer who had progressed after aromatase inhibitor therapy, the addition of selumetinib to fulvestrant did not improve survival compared to placebo plus fulvestrant[359]. Selumetinib has demonstrated efficacy in NF1-associated neurofibroma in Phase 2 studies[175,353-354] and a Phase 1 study[174]. Phase 2 studies reported clinical responses in low-grade glioma[178,360] , melanoma[361-365] , and in lung[85,182,366] and endometrial cancer[367]. A Phase 2 study of selumetinib for patients with activating alterations in the MAPK pathway reported a DCR of 15% (3/20), with no objective responses observed[368]. Phase 1 studies of selumetinib to treat patients with solid tumors reported 1/15 PR for a patient with colorectal cancer (CRC) and 5/15 SDs for patients with tonsil squamous cell carcinoma (SCC), non-small cell lung cancer (NSCLC), and CRC[369]; 2/39 PRs (for patients with CRC) and 18/39 SDs were achieved when selumetinib was administered in combination with cyclosporin A[370]. Multiple Phase 1 studies combining selumetinib with erlotinib or temsirolimus[371], docetaxel or dacarbazine[372], AKT inhibitors[373], or cixutumumab (an anti-IGF-1R antibody)[374] reported clinical responses for patients with advanced solid tumors including NSCLC, thyroid carcinoma, tongue SCC, and ovarian cancer.

Temsirolimus

Assay findings association

PIK3CA
E545K, E726K

AREAS OF THERAPEUTIC USE

Temsirolimus is an intravenous mTOR inhibitor that is FDA approved for the treatment of advanced renal cell carcinoma. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence[121-128] , PIK3CA activating mutations may predict sensitivity to mTOR inhibitors such as everolimus and temsirolimus. Studies have reported modest activity of these therapies as single agents (ORRs of 0-4%), but improved activity has been observed when they are combined with other agents such as bevacizumab and doxorubicin (ORRs of 25-44%), for patients with PIK3CA-mutated solid tumors[125-128,334-338] .

SUPPORTING DATA

Clinical benefit has been reported for patients with PIK3CA-mutated breast cancer treated with temsirolimus as a single agent[375] or in combination with doxorubicin and bevacizumab[127-128,337,376-378] . A Phase 1 trial examining the combination of temsirolimus, liposomal doxorubicin, and bevacizumab in 74 patients with breast and gynecological malignancies reported that 37.9% of patients experienced either a CR (1.4%), PR (18.9%), or SD (17.6%); among 25 patients with PIK3CA mutation or PTEN loss, 52% experienced a CR, PR (36%), or SD (16%)[128]. Another Phase 1 trial including patients with several types of cancer reported a 42% incidence of complete or partial responses in patients with metastatic breast cancer[126]. However, a Phase 2 study of temsirolimus in pretreated patients with metastatic breast cancer reported minimal clinical activity and no association with PTEN protein or PIK3CA mutation status[375]. A Phase 3 placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer was terminated at the second interim since the addition of temsirolimus to letrozole did not improve PFS as a first-line therapy[379]. A study examining the efficacy of temsirolimus-involving regimens in 24 patients with mesenchymal/metaplastic breast cancer (MpBCs) reported 2 CRs, 4 PRs, 2 instances of SD longer than 6 months, and 4 instances of SD shorter than 6 months[337].

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Trametinib

Assay findings association

NF1
G1092fs*14

AREAS OF THERAPEUTIC USE

Trametinib is a MEK inhibitor that is FDA approved as a monotherapy to treat patients with melanoma with BRAF V600E or V600K mutations. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence in neurofibromatosis type 1 (NF1)-associated neurofibroma[174-177,353-357], glioma[177-181,358], and non-small cell lung cancer[182], NF1 inactivation may predict sensitivity to MEK inhibitors.

SUPPORTING DATA

A Phase 1b trial of trametinib in combination with gemcitabine in patients with solid tumors showed a CR in a patient with breast cancer, as well as PRs in patients with pancreatic or salivary gland cancer[380]. Another patient with triple negative breast cancer reported a clinical response upon single-agent trametinib treatment[381]. A Phase 1 trial of trametinib in 206 patients with solid tumors reported 21 (10%) objective responses[382]. The MEK inhibitor PD184352 achieved SD in 1/14 patients (7%) with breast cancer[383]. Addition of the MEK inhibitor selumetinib to fulvestrant as second line therapy for patients who had progressed on aromatase inhibitor did not improve the DCR (23% vs. 50% with placebo) or the median PFS (3.7 months vs. 5.6 months with placebo) and was poorly tolerated[359]. Phase 1 monotherapy trials of RO4987655, another MEK inhibitor, have shown significant response rates in patients with melanoma, including those with BRAF and NRAS mutations, but very low response rates in patients with other solid tumors, including those with KRAS mutations[384-385]. A Phase 1b trial of combination treatment with the MEK inhibitor MEK162 and the PI3K-alpha inhibitor BYL719 reported disease control in 47% (21/45) of patients, including PRs in 2 of 3 patients with KRAS-mutant ovarian cancer and 1 of 3 patients with NRAS-mutant melanoma; a 43% rate of SD was observed in patients with KRAS-mutant colorectal cancer, with responses independent of PIK3CA mutation status[386]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[188], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[189].

NOTE Genomic alterations detected may be associated with activity of certain US FDA or other specific country approved therapies; however, the therapies listed in this report may have varied evidence in the patient's tumor type. The listed therapies are not ranked in order of potential or predicted efficacy for this patient or in order of level of evidence for this patient's tumor type. The therapies listed in this report may not be complete and/or exhaustive. Furthermore, the listed therapies are limited to US FDA approved pharmaceutical drug products that are linked to a specific genomic alteration. There may also be US FDA approved pharmaceutical drug products that are not linked to a genomic alteration. Further there may also exist pharmaceutical drug products that are not approved by the US FDA or other national authorities. There may also be other treatment modalities available than pharmaceutical drug products.

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IMPORTANT Clinical trials are ordered by gene and prioritized by: age range inclusion criteria for pediatric patients, proximity to ordering medical facility, later trial phase, and verification of trial information within the last two months. While every effort is made to ensure the accuracy of the information contained below, the information available in the public domain is continually updated and should be investigated by the physician or research staff. This is not a comprehensive list of all available clinical trials. There may also be compassionate use or early access programs available, which are not listed in this report. Foundation Medicine displays a subset of trial options and ranks them in this order of descending priority: Qualification for pediatric trial → Geographical proximity → Later trial phase. Clinical trials are not ranked in order of potential or predicted efficacy for this patient or in order of level of evidence for this patient's tumor type. Clinical trials listed here may have additional enrollment criteria that may require medical screening to determine final eligibility. For additional information about listed clinical trials or to conduct a search for additional trials, please see clinicaltrials.gov. However, clinicaltrials.gov does not list all clinical trials that might be available.

GENE
ERBB2

ALTERATION
A775_G776insYVMA

RATIONALE
ERBB2 amplification or activating mutation may confer sensitivity to HER2-targeted and dual EGFR/HER2-directed therapies, and may enhance efficacy of HSP90 inhibitors. Clinical and preclinical data suggest that ERBB2 exon 20 insertions confer resistance to lapatinib and reduced sensitivity to afatinib, dacomitinib, and neratinib. However, it is unclear if ERBB2 exon 20 insertions confer reduced sensitivity to lapatinib in combination with other therapies, such as trastuzumab. Retrospective clinical data suggest that ERBB2 A775_G776insYVMA is associated with inferior PFS with afatinib, compared with other ERBB2 mutations or exon 20 insertions. Investigational agents such as poziotinib and pyrotinib, or ERBB2-targeted antibodies such as trastuzumab and T-DM1, may be more effective.

NCT04494425

PHASE 3

Study of Trastuzumab Deruxtecan (T-DXd) vs Investigator's Choice Chemotherapy in HER2-low, Hormone Receptor Positive, Metastatic Breast Cancer

TARGETS
ERBB2

LOCATIONS: Sherbrooke (Canada), Massachusetts, Montreal (Canada), New York, New Jersey, Toronto (Canada), District of Columbia, Maryland

NCT02693535

PHASE 2

TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer

TARGETS
VEGFRs, ABL, SRC, ALK, ROS1, AXL, TRKA, MET, TRKC, CDK4, CDK6, FLT3, CSF1R, KIT, RET, mTOR, EGFR, ERBB2, MEK, BRAF, SMO, DDR2, PARP, PD-1, CTLA-4, ERBB4

LOCATIONS: Maine

NCT04589845

PHASE 2

Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, RET, PD-L1, AKTs, ERBB2, MDM2, PI3K-alpha

LOCATIONS: Maine, Seoul (Korea, Republic of), Montreal (Canada), New York, Ottawa (Canada), New Jersey, Pennsylvania, Delaware

NCT04579380

PHASE 2

Basket Study of Tucatinib and Trastuzumab in Solid Tumors With HER2 Alterations

TARGETS
ERBB2, ER

LOCATIONS: Massachusetts, Connecticut, New York, District of Columbia, Virginia, Pennsylvania, Ohio, North Carolina, South Carolina

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CLINICAL TRIALS

NCT04632992

PHASE 2

A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, PD-L1, ERBB2, PI3K-alpha, RET, AKTs

LOCATIONS: Connecticut, New York, New Jersey, Maryland, Virginia, Ohio, Michigan

NCT04644068

PHASE 1/2

Study of AZD5305 as Monotherapy and in Combination With Anti-cancer Agents in Patients With Advanced Solid Malignancies

TARGETS
ERBB2, TROP2, PARP

LOCATIONS: Montreal (Canada), New York, Toronto (Canada), Oklahoma, Texas, Manchester (United Kingdom), Oxford (United Kingdom), Cambridge (United Kingdom), Sutton (United Kingdom), Sevilla (Spain)

NCT03297606

PHASE 2

Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)

TARGETS
VEGFRs, ABL, SRC, ALK, ROS1, AXL, TRKA, MET, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, FLT3, CSF1R, RET, mTOR, ERBB2, MEK, BRAF, SMO

LOCATIONS: Montreal (Canada), Ottawa (Canada), Kingston (Canada), Toronto (Canada), London (Canada), Regina (Canada), Saskatoon (Canada), Edmonton (Canada), Vancouver (Canada)

NCT04556773

PHASE 1

A Phase 1b Study of T-DXd Combinations in HER2-low Advanced or Metastatic Breast Cancer

TARGETS
PD-L1, ERBB2, AKTs, Aromatase

LOCATIONS: Quebec (Canada), New York, New Jersey, North Carolina, Tennessee, Minnesota

NCT04042701

PHASE 1

DS8201a and Pembrolizumab in Participants With Locally Advanced/Metastatic Breast or Non-Small Cell Lung Cancer

TARGETS
PD-1, ERBB2

LOCATIONS: Massachusetts, Pennsylvania, Maryland, Florida, Texas, London (United Kingdom), Sutton (United Kingdom), Madrid (Spain), Bordeaux (France), California

NCT04539938

PHASE 2

A Study of Tucatinib Plus Trastuzumab Deruxtecan in HER2+ Breast Cancer

TARGETS
ERBB2

LOCATIONS: Massachusetts, New York, New Jersey, District of Columbia, Virginia, Pennsylvania, North Carolina, Kentucky, Wisconsin

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GENE
NF1

ALTERATION
G1092fs*14

RATIONALE
On the basis of clinical evidence and strong preclinical evidence, NF1 inactivation may predict sensitivity to MEK inhibitors. Limited clinical data and strong preclinical data indicate that loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors.

NCT03284957

PHASE 1/2

Phase 1 / 2 Study of SAR439859 Single Agent and in Combination With Palbociclib in Postmenopausal Women With Estrogen Receptor Positive Advanced Breast Cancer

TARGETS
ER, CDK4, CDK6, PI3K-alpha, mTOR

LOCATIONS: Massachusetts, New York, Toronto (Canada), Saint-Herblain (France), Colorado, Glasgow (United Kingdom), Porto (Portugal), Vancouver (Canada), Washington, Lisboa (Portugal)

NCT04802759

PHASE 1/2

A Study Evaluating the Efficacy and Safety of Multiple Treatment Combinations in Participants With Breast Cancer

TARGETS
ER, CDK4, CDK6, AKTs, PI3K-alpha, mTOR

LOCATIONS: Massachusetts, New Jersey, Pennsylvania, North Carolina, Tennessee, California, Madrid (Spain)

NCT03297606

PHASE 2

Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)

TARGETS
VEGFRs, ABL, SRC, ALK, ROS1, AXL, TRKA, MET, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, FLT3, CSF1R, RET, mTOR, ERBB2, MEK, BRAF, SMO

LOCATIONS: Montreal (Canada), Ottawa (Canada), Kingston (Canada), Toronto (Canada), London (Canada), Regina (Canada), Saskatoon (Canada), Edmonton (Canada), Vancouver (Canada)

NCT04188548

PHASE 1

A Study of LY3484356 in Participants With Advanced or Metastatic Breast Cancer or Endometrial Cancer

TARGETS
mTOR, Aromatase, CDK4, CDK6, ER, PI3K-alpha, ERBB2

LOCATIONS: Massachusetts, Vermont, New York, Maryland, Virginia, Pennsylvania, North Carolina

NCT03971409

PHASE 2

Avelumab With Binimetinib, Utomilumab, or Anti-OX40 Antibody PF-04518600 in Treating Triple Negative Breast Cancer

TARGETS
OX40, PD-L1, CD137, MEK

LOCATIONS: District of Columbia, Alabama, Texas, California

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CLINICAL TRIALS

NCT05054374

PHASE 1/2

A Study of Mirdametinib on Its Own or in Combination With Fulvestrant in People With Solid Tumor Cancer

TARGETS
MEK, ER

LOCATIONS: New York, New Jersey

NCT02531932

PHASE 2

Comparison of Single-Agent Carboplatin vs the Combination of Carboplatin and Everolimus for the Treatment of Advanced Triple-Negative Breast Cancer

TARGETS
mTOR

LOCATIONS: New York

NCT03032406

PHASE 2

CLEVER Pilot Trial: A Phase II Pilot Trial of HydroxyChLoroquine, EVErolimus or the Combination for Prevention of Recurrent Breast Cancer

TARGETS
mTOR

LOCATIONS: Pennsylvania

NCT04800822

PHASE 1

PF-07284892 in Participants With Advanced Solid Tumors

TARGETS
SHP2, ROS1, ALK, MEK, BRAF, EGFR

LOCATIONS: New York, Michigan, Tennessee, Texas, California

NCT04683354

PHASE 1

Study of HL-085 in Patients With Advanced Solid Tumor Tumors

TARGETS
MEK

LOCATIONS: Ohio, Tennessee, Texas, Nevada, California

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GENE
PIK3CA

ALTERATION
E545K, E726K

RATIONALE
PIK3CA activating mutations may lead to activation of the PI3K-AKT-mTOR pathway and may therefore indicate sensitivity to inhibitors of this pathway. Strong clinical data support sensitivity of PIK3CA-mutated solid tumors to the PI3K-alpha inhibitor alpelisib.

NCT04650581

PHASE 3

Fulvestrant and Ipatasertib for Advanced HER-2 Negative and Estrogen Receptor Positive (ER+) Breast Cancer Following Progression on First Line CDK 4/6 Inhibitor and Aromatase Inhibitor

TARGETS
ER, AKTs

LOCATIONS: Halifax (Canada), Saint John (Canada), Quebec City (Canada), Greenfield Park (Canada), Montreal (Canada), Ottawa (Canada), Kingston (Canada), Newmarket (Canada), Toronto (Canada), Barrie (Canada)

NCT04191499

PHASE 2/3

A Study Evaluating the Efficacy and Safety of GDC-0077 + Palbociclib + Fulvestrant vs Placebo + Palbociclib + Fulvestrant in Patients With PIK3CA-Mutant, Hormone Receptor-Positive, Her2-Negative, Locally Advanced or Metastatic Breast Cancer

TARGETS
PI3K-alpha, CDK6, ER, CDK4

LOCATIONS: Rimouski (Canada), Quebec City (Canada), Massachusetts, Montreal (Canada), Ankara (Turkey), Ottawa (Canada), New York, Barrie (Canada), North Carolina, Georgia

NCT04862663

PHASE 3

Capivasertib + Palbociclib + Fulvestrant for HR+/HER2- Advanced Breast Cancer (CAPItello-292).

TARGETS
AKTs, CDK6, ER, CDK4

LOCATIONS: Chicoutimi (Canada), St Herblain (France), Tennessee, Colorado, Texas, Villejuif (France), Leuven (Belgium), Odense C (Denmark), Solna (Sweden), Bydgoszcz (Poland)

NCT04589845

PHASE 2

Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, RET, PD-L1, AKTs, ERBB2, MDM2, PI3K-alpha

LOCATIONS: Maine, Seoul (Korea, Republic of), Montreal (Canada), New York, Ottawa (Canada), New Jersey, Pennsylvania, Delaware

NCT03284957

PHASE 1/2

Phase 1 / 2 Study of SAR439859 Single Agent and in Combination With Palbociclib in Postmenopausal Women With Estrogen Receptor Positive Advanced Breast Cancer

TARGETS
ER, CDK4, CDK6, PI3K-alpha, mTOR

LOCATIONS: Massachusetts, New York, Toronto (Canada), Saint-Herblain (France), Colorado, Glasgow (United Kingdom), Porto (Portugal), Vancouver (Canada), Washington, Lisboa (Portugal)

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CLINICAL TRIALS

NCT03994796

PHASE 2

Genetic Testing in Guiding Treatment for Patients With Brain Metastases

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, CDK4, CDK6, PI3K, mTOR

LOCATIONS: Massachusetts, New Hampshire, Vermont, Connecticut, New Jersey, Pennsylvania

NCT04802759

PHASE 1/2

A Study Evaluating the Efficacy and Safety of Multiple Treatment Combinations in Participants With Breast Cancer

TARGETS
ER, CDK4, CDK6, AKTs, PI3K-alpha, mTOR

LOCATIONS: Massachusetts, New Jersey, Pennsylvania, North Carolina, Tennessee, California, Madrid (Spain)

NCT04317105

PHASE 1/2

Testing the Addition of an Anti-cancer Drug, Copanlisib, to the Usual Immunotherapy (Nivolumab With or Without Ipilimumab) in Patients With Advanced Solid Cancers That Have Changes in the Following Genes: PIK3CA and PTEN

TARGETS
PD-1, CTLA-4, PI3K

LOCATIONS: Massachusetts, Toronto (Canada), Virginia, Texas

NCT04632992

PHASE 2

A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, PD-L1, ERBB2, PI3K-alpha, RET, AKTs

LOCATIONS: Connecticut, New York, New Jersey, Maryland, Virginia, Ohio, Michigan

NCT03297606

PHASE 2

Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)

TARGETS
VEGFRs, ABL, SRC, ALK, ROS1, AXL, TRKA, MET, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, FLT3, CSF1R, RET, mTOR, ERBB2, MEK, BRAF, SMO

LOCATIONS: Montreal (Canada), Ottawa (Canada), Kingston (Canada), Toronto (Canada), London (Canada), Regina (Canada), Saskatoon (Canada), Edmonton (Canada), Vancouver (Canada)

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NOTE One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

ATRX Y2176F	***DNMT3A*** T437R	***KDM5C*** S396N	***NTRK1*** G18E
PBRM1 R1540C	***RET*** L56M	***WHSC1 (MMSET)*** Q295L	***ZNF217*** S329F

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APPENDIX Genes assayed in FoundationOne®Liquid CDx

FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *) ; 75 genes (indicated in bold) are captured with increased sensitivity and have complete exonic (coding) coverage unless otherwise noted.

ABL1 **Exons 4-9**	*ACVR1B*	***AKT1*** **Exon 3**	*AKT2*	*AKT3*	***ALK*** **Exons 20-29, Introns 18, 19**	*ALOX12B*	*AMER1* (FAM123B or WTX)	***APC***
AR	***ARAF*** **Exons 4, 5, 7, 11, 13, 15, 16**	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*
BCOR	*BCORL1*	*BCR** Introns 8, 13, 14	***BRAF*** **Exons 11-18,** Introns 7-10	***BRCA1*** Introns 2, 7, 8, 12, 16, 19, 20	***BRCA2*** Intron 2	*BRD4*	*BRIP1*	*BTG1*
BTG2	***BTK*** **Exons 2, 15**	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*
CCND3	*CCNE1*	*CD22*	*CD70*	*CD74** Introns 6-8	*CD79A*	*CD79B*	***CD274*** **(PD-L1)**	*CDC73*
CDH1	***CDK12***	***CDK4***	***CDK6***	*CDK8*	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*
CDKN2C	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*	*CREBBP*	***CRKL***	*CSF1R*	*CSF3R*
CTCF	*CTNNA1*	***CTNNB1*** **Exon 3**	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*
DDR2 **Exons 5, 17, 18**	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR*** **Introns 7,** 15**, 24-27**	*EMSY* (C11orf30)	*EP300*	*EPHA3*
EPHB1	*EPHB4*	***ERBB2***	***ERBB3*** **Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25**	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1*** **Exons 4-8**
*ETV4** Intron 8	*ETV5** Introns 6, 7	***ETV6**** **Introns 5, 6**	*EWSR1** Introns 7-13	***EZH2*** **Exons 4, 16, 17, 18**	*EZR** Introns 9-11	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	***FGFR1*** Introns 1, 5, **Intron 17**	***FGFR2*** Intron 1, **Intron 17**	***FGFR3*** **Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17**	*FGFR4*	*FH*	*FLCN*	*FLT1*
FLT3 **Exons 14, 15, 20**	***FOXL2***	*FUBP1*	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	*GID4* (C17orf39)	***GNA11*** **Exons 4, 5**
GNA13	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*	*GSK3B*	*H3-3A* (H3F3A)	*HDAC1*	*HGF*	*HNF1A*
HRAS **Exons 2, 3**	*HSD3B1*	*ID3*	***IDH1*** **Exon 4**	***IDH2*** **Exon 4**	*IGF1R*	*IKBKE*	*IKZF1*	*INPP4B*
IRF2	*IRF4*	*IRS2*	*JAK1*	***JAK2*** **Exon 14**	***JAK3*** **Exons 5, 11, 12, 13, 15, 16**	*JUN*	*KDM5A*	*KDM5C*
KDM6A	*KDR*	*KEAP1*	*KEL*	***KIT*** **Exons 8, 9, 11, 12, 13, 17,** Intron 16	*KLHL6*	*KMT2A* (MLL) Introns 6, 8-11, Intron 7	*KMT2D* (MLL2)	

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APPENDIX Genes assayed in FoundationOne®Liquid CDx

FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *) ; 75 genes (indicated in bold) are captured with increased sensitivity and have complete exonic (coding) coverage unless otherwise noted.

KRAS	*LTK*	*LYN*	*MAF*	***MAP2K1*** **(MEK1) Exons 2, 3**	***MAP2K2*** **(MEK2) Exons 2-4, 6, 7**	*MAP2K4*	*MAP3K1*	*MAP3K13*
MAPK1	*MCL1*	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***
MITF	*MKNK1*	*MLH1*	***MPL*** **Exon 10**	*MRE11* (MRE11A)	*MSH2* Intron 5	*MSH3*	*MSH6*	*MST1R*
MTAP	***MTOR*** **Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56**	*MUTYH*	*MYB** Intron 14	***MYC*** **Intron 1**	*MYCL* (MYCL1)	***MYCN***	***MYD88*** **Exon 4**	*NBN*
NF1	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2* Intron 26	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**
NRAS **Exons 2, 3**	*NSD2* (WHSC1 or MMSET)	*NSD3* (WHSC1L1)	*NT5C2*	***NTRK1*** **Exons 14, 15, Introns 8-11**	*NTRK2* Intron 12	***NTRK3*** **Exons 16, 17**	*NUTM1** Intron 1	*P2RY8*
PALB2	*PARP1*	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1* (PD-1)	***PDCD1LG2*** **(PD-L2)**	***PDGFRA*** **Exons 12, 18, Introns 7, 9, 11**
PDGFRB **Exons 12-21, 23**	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA*** **Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)**	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*
POLD1	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PRKN* (PARK2)
PTCH1	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1*** **Exons 3, 4, 6, 7, 10, 14, 15, 17,** Introns 4-8	*RARA* Intron 2	***RB1***	*RBM10*	*REL*
RET Introns 7, 8, **Exons 11, 13-16, Introns 9-11**	*RICTOR*	*RNF43*	***ROS1*** **Exons 31, 36-38, 40, Introns 31-35**	*RPTOR*	*RSPO2** Intron 1	*SDC4** Intron 2	*SDHA*	*SDHB*
SDHC	*SDHD*	*SETD2*	*SF3B1*	*SGK1*	*SLC34A2** Intron 4	*SMAD2*	*SMAD4*	*SMARCA4*
SMARCB1	***SMO***	*SNCAIP*	*SOCS1*	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*
STAG2	*STAT3*	***STK11***	*SUFU*	*SYK*	*TBX3*	*TEK*	*TENT5C* (FAM46C)	*TERC** ncRNA
TERT* **Promoter**	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2** Introns 1-3	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*
TSC2	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WT1*	*XPO1*	*XRCC2*	*ZNF217*
ZNF703								

ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER BIOMARKERS

Microsatellite (MS) status
Blood Tumor Mutational Burden (bTMB)
Tumor Fraction

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FoundationOne Liquid CDx fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices and is registered as a CE-IVD product by Foundation Medicine's EU Authorized Representative, Qarad b.v.b.a, Cipalstraat 3, 2440 Geel, Belgium. The CE-IVD regulatory status of FoundationOne Liquid CDx is applicable in countries that accept and/or recognize the CE mark.

CE IVD

ABOUT FOUNDATIONONE LIQUID CDx

FoundationOne Liquid CDx was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne Liquid CDx may be used for clinical purposes and should not be regarded as purely investigational or for research only. Foundation Medicine's clinical reference laboratories are qualified to perform high-complexity clinical testing.

Please refer to technical information for performance specification details.

INTENDED USE

FoundationOne Liquid CDx is a next generation sequencing based *in vitro* diagnostic device that analyzes 324 genes. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The test also detects the genomic signatures blood tumor mutational burden (bTMB), microsatellite instability (MSI), and tumor fraction. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from the anti-coagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with targeted therapies in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms.

TEST PRINCIPLES

The FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes including coding exons and select introns of 309 genes, as well as only select intronic regions or non-coding regions of 15 genes. Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a customized analysis pipeline designed to accurately detect genomic alterations, including base substitutions, indels, select copy number variants, and select genomic rearrangements. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The assay also reports tumor fraction, and genomic signatures including MSI and bTMB. A subset of targeted regions in 75 genes is baited for increased sensitivity.

THE REPORT

Incorporates analyses of peer-reviewed studies and other publicly available information identified by Foundation Medicine; these analyses and information may include associations between a molecular alteration (or lack of alteration) and one or more drugs with potential clinical benefit (or potential lack of clinical benefit), including drug candidates that are being studied in clinical research. *Note:* A finding of biomarker alteration does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen.

QUALIFIED ALTERATION CALLS (EQUIVOCAL)

All equivocal calls, regardless of alteration type, imply that there is adequate evidence to call the alteration with confidence. However, the repeatability of equivocal calls may be lower than non-equivocal calls.

RANKING OF THERAPIES AND CLINICAL TRIALS

Ranking of Therapies in Summary Table
Therapies are ranked based on the following criteria: Therapies with clinical benefit (ranked alphabetically within each evidence category), followed by therapies associated with resistance (when applicable).

Ranking of Clinical Trials
Pediatric trial qualification → Geographical proximity → Later trial phase.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. A negative result does not rule out the presence of a mutation below the limits of detection of the assay. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an appropriately validated tumor tissue test, if available.
4. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
5. The test is not intended to provide information on cancer predisposition.
6. Performance has not been validated for cfDNA input below the specified minimum input.
7. Tissue TMB and blood TMB (bTMB) are estimated from the number of synonymous and nonsynonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of ≥5%, and bTMB is calculated based on variants with an allele frequency of ≥0.5%.
8. Tumor fraction is the percentage of circulating tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample. The tumor fraction estimate is computationally derived from the observed level of aneuploidy in the sample. Tumor fraction is considered elevated when ctDNA levels are high enough that aneuploidy can be detected and is significantly distinct from that typically found in non-tumor samples.
9. Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the tumor genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor. The MSI algorithm is based on genome wide analysis of 1765 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines for solid tissue testing.
10. Genomic findings from circulating cell-free DNA (cfDNA) may originate from circulating tumor DNA fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited

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to: *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *JAK2*, *KMT2D* (*MLL2*), *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*.

11. Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
12. The test is not intended to replace germline testing or to provide information about cancer predisposition.

REPORT HIGHLIGHTS

The Report Highlights includes select genomic and therapeutic information with potential impact on patient care and treatment that is specific to the genomics and tumor type of the sample analyzed. This section may highlight information including targeted therapies with potential sensitivity or resistance; evidence-matched clinical trials; and variants with potential diagnostic, prognostic, nontargeted treatment, germline, or clonal hematopoiesis implications. Information included in the Report Highlights is expected to evolve with advances in scientific and clinical research. Findings included in the Report Highlights should be considered in the context of all other information in this report and other relevant patient information. Decisions on patient care and treatment are the responsibility of the treating physician.

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING

The variants indicated for consideration of follow-up germline testing are 1) limited to reportable short variants with a protein effect listed in the ClinVar genomic database (Landrum et al., 2018; 29165669) as Pathogenic, Pathogenic/Likely Pathogenic, or Likely Pathogenic (by an expert panel or multiple submitters), 2) associated with hereditary cancer-predisposing disorder(s), 3) detected at an allele frequency of >30%, and 4) in select genes reported by the ESMO Precision Medicine Working Group (Mandelker et al., 2019; 31050713) to have a greater than 10% probability of germline origin if identified during tumor sequencing. The selected genes are *ATM, BAP1, BRCA1, BRCA2, BRIP1, CHEK2, FH, FLCN, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, POLE, RAD51C, RAD51D, RET, SDHA, SDHB, SDHC, SDHD, TSC2,* and *VHL*, and are not inclusive of all cancer susceptibility genes. The content in this report should not substitute for genetic counseling or follow-up germline testing, which is needed to distinguish whether a finding in this patient's tumor sequencing is germline or somatic. Interpretation should be based on clinical context.

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS

Variants that may represent clonal hematopoiesis (CH) are limited to select reportable short variants in defined genes identified in solid tumors only. Variant selection was determined based on gene tumor-suppressor or oncogene status, known role in solid tumors versus hematological malignancies, and literature prevalence. The defined genes are *ASXL1, ATM, CBL, CHEK2, DNMT3A, IDH2, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2,* and *U2AF1* and are not inclusive of all CH genes. The content in this report should not substitute for dedicated hematological workup. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH. Interpretation should be based on clinical context.

NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®) CATEGORIZATION

Biomarker and genomic findings detected may be associated with certain entries within the NCCN Drugs & Biologics Compendium® (NCCN Compendium®) (www.nccn.org). The NCCN Categories of Evidence and Consensus indicated reflect the highest possible category for a given therapy in association with each biomarker or genomic finding. Please note, however, that the accuracy and applicability of these NCCN categories within a report may be impacted by the patient's clinical history, additional biomarker information, age, and/or co-occurring alterations. For additional information on the NCCN categories, please refer to the NCCN Compendium®. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. To view the most recent and complete version of the guidelines, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

LEVEL OF EVIDENCE NOT PROVIDED

Drugs with potential clinical benefit (or potential lack of clinical benefit) are not evaluated for source or level of published evidence.

NO GUARANTEE OF CLINICAL BENEFIT

This report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

NO GUARANTEE OF REIMBURSEMENT

Foundation Medicine makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of FoundationOne Liquid CDx.

TREATMENT DECISIONS ARE THE RESPONSIBILITY OF PHYSICIAN

Drugs referenced in this Report may not be suitable for a particular patient. The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides entirely within the discretion of the treating physician. Indeed, the information in this Report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as this test or the information contained in this report.

Certain sample of variant characteristics may result in reduced sensitivity. These include: low sample quality, deletions and insertions >40bp, or repetitive/high homology sequences. FoundationOne Liquid CDx is performed using cell-free DNA, and as such germline events may not be reported.

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SELECT ABBREVIATIONS

ABBREVIATION	DEFINITION
CR	Complete response
DCR	Disease control rate
DNMT	DNA methyltransferase
HR	Hazard ratio
ITD	Internal tandem duplication
MMR	Mismatch repair
Muts/Mb	Mutations per megabase
NOS	Not otherwise specified
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
SD	Stable disease
TKI	Tyrosine kinase inhibitor

REFERENCE SEQUENCE INFORMATION

Sequence data is mapped to the human genome, Genome Reference Consortium Human Build 37 (GRCh37), also known as hg19.

MR Suite Version 6.3.0

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